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Development of AB<sub>1-42</sub> assay

An assay to ~~for~~ measure AB<sub>1-42</sub> has been developed

Plates were coated with 50 µl and fixed with 0.25% HSA

Samples / calibrators 1-42 0.125 - 10 µg/ml were added, 100 µl / well and incubated for 1h at RT

The super was aspirated. Plates washed 3x and alkaline 277-2, ~~anti~~ rabbit polyclonal antibody, raised against ~~the~~ last 10 amino acids of AB<sub>32-42</sub>, added at 1 µg/ml in conjugate diluent, 100 µl / well, and incubated at RT for 1h

Fluid is aspirated and washed 3x. Alkaline phosphatase conjugated anti-donkey anti-rabbit mouse absorbed diluted 1:1000 in conjugate diluent was added, 100 µl / well, and incubated for 1h at RT

Sup. aspirated, plates washed 3x

Fluorescent substrate MUP added 100 µl / well and incubated for 1h at RT. 15 min at RT. Plates read

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Proteolytic activity of AB<sub>1-42</sub> with AB<sub>1-40</sub>, AB<sub>1-38</sub>, and AB<sub>1-28</sub> was studied. The effect of triton, NP40 and octylglucoside, NP40 and SDS and boiling was studied.

Plate Map 1



	Date tested											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.625	1.25	2.5	5.0	10.0	B						
B												
C												
D												
E												
F												
G												
H												

← AB<sub>1-42</sub>

← AB<sub>1-40</sub>

← AB<sub>1-38</sub>

← AB<sub>1-28</sub>

} Same + 1% triton

Plate #2 Same as 1 but Rows E-F with (CV 2% triton x 100) 0.2% NP40.

Plate #3 Same as 1, Rows E-F AB<sub>1-42</sub> boiled

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A<sub>3</sub> 1-42

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	1	2	3	4	5	6	7	8	9	10	11	12
A Set 1	896	742	1386	1390	1867	1820	2089	2037	2167	2161	158	175
B Set 1	105	98	98	156	153	111	187	139	221	165	199	223
C Set 1	98	100	83	110	103	121	113	95	97	97	99	108
D Set 1	97	97	84	121	113	122	135	100	95	105	114	107
E Set 1	381	302	604	540	1052	945	1466	1474	1825	1841	103	111
F Set 1	116	93	93	124	108	126	144	91	99	111	110	136
G Set 1	122	126	203	121	114	124	179	124	93	102	162	145
H Set 1	77	73	77	101	128	123	103	108	100	100	136	161

	1	2	3	plate #2	5	6	7	8	9	10	11	12
A Set 1	684	636	1119	1174	1682	1654	1958	1964	2083	2119	117	110
B Set 1	135	146	111	139	124	131	147	119	120	135	120	139
C Set 1	123	108	98	98	120	115	120	122	134	125	115	125
D Set 1	102	89	90	92	92	89	106	102	89	100	107	118
E Set 1	378	222	455	502	940	1006	1397	1366	1564	1586	158	231
F Set 1	88	93	87	108	112	87	97	96	90	97	129	120
G Set 1	125	114	93	138	150	129	174	185	111	116	125	109
H Set 1	64	61	60	64	68	119	121	83	82	83	121	143

		1	2	3	4	5	6	7	8	9	10	11	12
A Set	1	plate #3 1085	889	914	1049	1508	1617	2031	1904	2210	2204	232	207
B Set	1	256	222	188	176	176	171	255	257	198	246	247	218
C Set	1	260	191	309	355	322	304	346	270	309	305	261	281
D Set	1	176	165	171	162	174	205	279	221	213	195	232	239
E Set	1	383	368	562	622	1110	922	1678	1631	1958	1986	210	218
F Set	1	202	252	196	221	255	245	280	316	332	346	209	246
G Set	1	250	203	132	169	210	229	279	261	277	337	230	351
H Set	1	213	186	194	217	224	271	278	299	285	311	205	205

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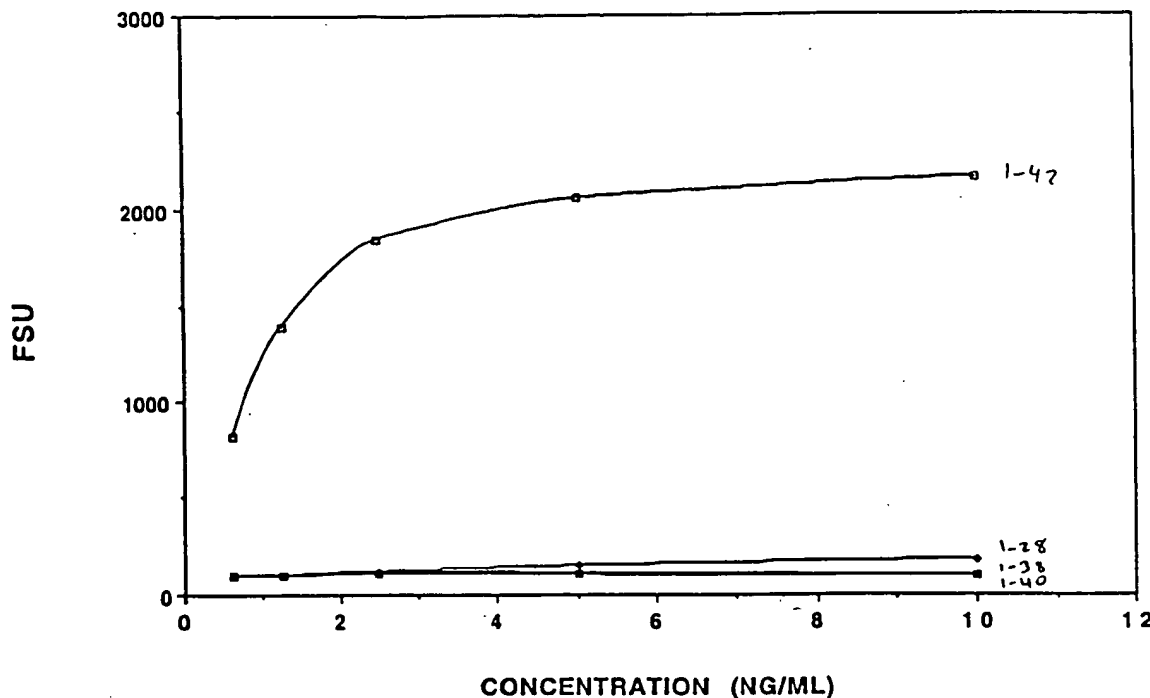
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PROJECT

AB 1-42

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AB 1-42, 1-40, 1-38, 1-28 STD CURVE (266-272)



This assay detects AB-42 immunoreactivity with sensitivity greater than 0.625 and with no cross-reactivity with AB-40, 1-38 or AB-28.

There is little effect of Triton X-100, NP-40 or bovine serum albumin. This can have relevant consequences in sample assay, CSF in particular.

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Effect of Triton X-100, NP-40 and boiling  
on AP<sub>1</sub>-42 std. curve and two CSF  
samples

## Plate Map

athena neurosciences

266-272-2

Date Tested 11/17/93

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.125	0.625	1.25	2.5	5.0	PB						
B												
C												
D												
E	12/12 heat	12/12 Boiled	12/12 T	12/12 NP40	1406 heat	1406 Boiled						
F	1406 T	1406 NP40	1406 PB	1406 PB	1406 PB							
G	1406 PB	1406 PB	1406 PB	1406 PB	1406 PB							
H	1406 PB	1406 PB	1406 PB	1406 PB	1406 PB							

← Boiled  
← T  
← NP40

The assay was performed as described in p 52

	1	2	3	4	5	6	7	8	9	10	11	12
A Set 1	217	203	997	1009	1604	1582	1997	1958	2161	2167	90	83
B Set 1	173	158	417	489	838	914	1503	1433	1820	1920	102	90
C Set 1	154	141	407	432	833	842	1425	1235	1721	1750	112	109
D Set 1	139	145	456	530	997	1049	1516	1314	1915	1936	107	109
E Set 1	350	364	424	394	167	190	216	215	740	746	978	994
F Set 1	332	296	294	278	124	116	120	114	105	97	91	95
G Set 1	282	279	141	139	150	142	134	132	128	117	117	117
H Set 1	126	128	127	127	132	127	123	126	118	110	114	133

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